IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES OF SORGHUM [*SORGHUM BICOLOR* (L.) MOENCH] SEEDLINGS UNDER NITROGEN STRESS BY RNA-SEQ

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Abstract. Sorghum [Sorghum bicolor (L.) Moench] is an important cereal crop with high nitrogen utilization efficiency. In order to analyze the mechanisms of different sorghum genotypes to low-N tolerance and provide the theoretical basis for the breeding of low nitrogen tolerant sorghum varieties, two materials (KZ15 and SZ7) with different tolerance to low-N were treated with nitrogen by hydroponics, the phenotypes were observed, physiological indexes were measured and transcripts were analyzed in this study. Compared to SZ7, KZ15 showed significantly higher capacity of low-N tolerance. The results showed that the change of KZ15 chlorophyll content was lower than that of SZ7 under low-N stress. The activity of nitrate reductase (NR) and glutamine synthetase (GS) had a smaller reduction in KZ15 than SZ7 under low-N stress. High-throughput sequencing revealed several common differentially expressed genes (DEGs) between KZ15 and SZ7, including nitrogen transport and nitrate assimilation genes. In the tolerant genotype KZ15, there were more transcripts related to high affinity nitrate transporters (NRT2.4, NRT3.1 and NRT4.5), suggesting an improved uptake efficiency of inorganic and organic forms of nitrogen. In addition, an abundance of certain transcription factors in the tolerant genotype KZ15 suggests that they may play roles in regulating the response to N-stress. This study will enable us to discover candidate genes potentially useful in improving the resistance to low nitrogen stress by genetic engineering technology and provide theoretical basis for the breeding of low nitrogen tolerant sorghum varieties.
Keywords: *sorghum, N tolerance, chlorophyll, transcriptome, differentially expressed genes, candidate genes*

Introduction

Nitrogen is a necessary major element for the growth and development of higher plants, which takes part in a series of physiological and biochemical reactions,

involving various metabolic pathways (Kraiser et al., 2011). There are two mechanisms for obtaining nitrogen in plants: one is to absorb nitrogen nutrition by nodular nitrogenase, which is mainly in leguminous plants. The other is to absorb NH_4^+, NO_3^-, and other mineral ions from the soil by root transporters. Sorghum mainly absorbs nitrogen through the second mechanism. Studies of nitrogen stress by quantitative trait loci analysis have mainly focused on maize (Gallais et al., 2004), rice (Obara et al., 2004), Arabidopsis (Loudet et al., 2003a,b) and sorghum (Gelli et al., 2017). At present, the study in low nitrogen tolerance of sorghum has lagged behind other grain crops (Rosa et al., 2014). Few studies of the answers of the molecular response mechanisms of growth and development to nitrogen deficiency of sorghum have been reported.

In this study, the phenotypic and transcriptomic responses of two sorghum varieties, KZ15 and SZ7, under low nitrogen stress and control were investigated. Unraveling mechanisms underlying low nitrogen tolerance of sorghum may provide useful information to genetic engineering and improve the low nitrogen tolerance of sorghum. Such knowledge can also be useful for increasing nitrogen use efficiency for sustainable agriculture.

Materials and methods

Plant materials and growth conditions

Two sorghum varieties, Keza15 (KZ15, tolerant to nitrogen starvation) and Suiza7 (SZ7, sensitive to nitrogen starvation), were used for this study, which were provided by the Keshan branch of the Heilongjiang Academy of Agricultural Sciences.

This experiment was conducted in the greenhouse of Keshan Branch of Heilongjiang Academy of Agricultural Sciences in China. Uniform and plump-eared sorghum seeds were selected and germinated under dark conditions after disinfection, and then sown in vermiculite.

Stress treatments and sample preparations

Trifoliate seedlings were moved into foam boxes wrapped in black plastic film and then cultured with Hoagland nutrient solution. The average temperature and light-dark period were 25°C/20°C and 14 h/10 h. $CaCl_2$ was added to supplement Ca^{2+}. Control plants (in the solution of 4 mmol/L $Ca(NO_3)_2$) were grown in parallel and collected at the same time points. The pH value was adjusted to 5.8 (Ma, 2014). KA, SA, KB and SB indicated the seedlings of KZ15 and SZ7 in low nitrogen treatment and control, respectively. The process was repeated in triplicate. Leaves of seedling which treated for seven days were taken as samples.

The method of the determination of chlorophyll content was referred to the modified method cited by Wang et al. (2006). The following formulas were used to calculate the content of photosynthetic pigments, in which V and W indicated the volume of extracting liquid (ml) and the weight of material (g).

$$\text{Chlorophyll a (Ca) / mg·g-1} = (12.21A663 - 2.81A646) \, V / (1000 \times W) \quad \text{(Eq.1)}$$

$$\text{Chlorophyll b (Cb) / mg·g-1} = (20.13A646 - 5.03A663) \, V / (1000 \times W) \quad \text{(Eq.2)}$$

The method of nitrate reductase determination was referred to Rajasekhar et al. (1986). The determination of glutamine synthase (GS) was referred to the method of Ding et al. (2006).

The experiment was performed using three biological replicates, and differences were statistically analyzed using Student's t-test ([*]P<0.05, [**]P<0.01). Bars indicate standard error of the mean. Microsoft Excel 2010 and SPSS 18.0 software are employed for data collation and analysis.

RNA seq analysis

Total RNA was isolated from each sample. Quality and quantity evaluation of total RNA, library constructing, and Illumina sequencing were performed in the Beijing Genome Institute (BGI, Shenzhen, China).

Transcript data analysis

TopHat 2 (Kim et al., 2015) was used to compare transcripts with reference genomes. After genome comparison, the StringTie tool was used to identify exonic regions, and then the transcript was reconstructed. The transcript was reconstructed by the DeNovo assembly method. The actual transcripts were screened out, and the low expression transcripts were filtered by the TPM value (filtering criteria: TPM > 0.5 in at least one sample).

Differential expressed genes between samples were defined by DESeq using two separate models (Anders and Huber, 2010), based on fold change >2 and false discovery rate–adjusted P value < 0.05.

According to the result of GO annotation, DEGs were classified to GO terms according to official classification. Phyper was used to perform GO functional enrichment. The p-value calculating formula in hyper geometric test was:

$$P = 1 - \sum_{i=0}^{m-1} \frac{\binom{M}{i}\binom{N-M}{n-i}}{\binom{N}{n}}$$

(Eq.3)

Then, false discovery rate (FDR) was calculated for each p-value. In general, the term FDR <= 0.01 was defined as significantly enriched.

Results

Phenotypic investigation of KZ15 and SZ7 under low-N stress

To evaluate the impact of low-N stress on sorghum, seedlings were planted in an intelligent greenhouse with low-N stress (0 mmol/L) and under control conditions (4 mmol/L). Under low-N stress, the seedlings of SZ7 leaves turned pale yellow, but the seedlings of KZ15 leaves did not significantly change (*Fig. 1A and 1B*). Subsequently, chlorophyll content, activity of nitrate reductase (NR) and glutamine synthetase (GS) were measured in both KZ15 and SZ7 of the low-N treatment and control. The results showed that the seedlings of SZ7 have a 28.77% average decrease in total chlorophyll content, but the seedlings of KZ15 only have 12.39% average decrease (*Fig. 1C*).

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Compared to the control, the content of chlorophyll a and chlorophyll b in SZ7 showed a 27.32% and 33.66% decrease, respectively. But the content of that in KZ15 was 8.86% and 22.71%, respectively (*Fig. 1D, 1E*). These results demonstrate that the change of KZ15 chlorophyll content was lower than that of SZ7 under low-N stress, and chlorophyll b content was more sensitive under low-N stress than the content of chlorophyll a. In addition, the activity of NR and GS was significantly reduced in SZ7 under low-N stress, but the activity of NR and GS exhibited little change in KZ15 under low-N stress (*Fig. 1F, 1G*). These results demonstrate that KZ15 is more N-stress tolerant compared with SZ7.
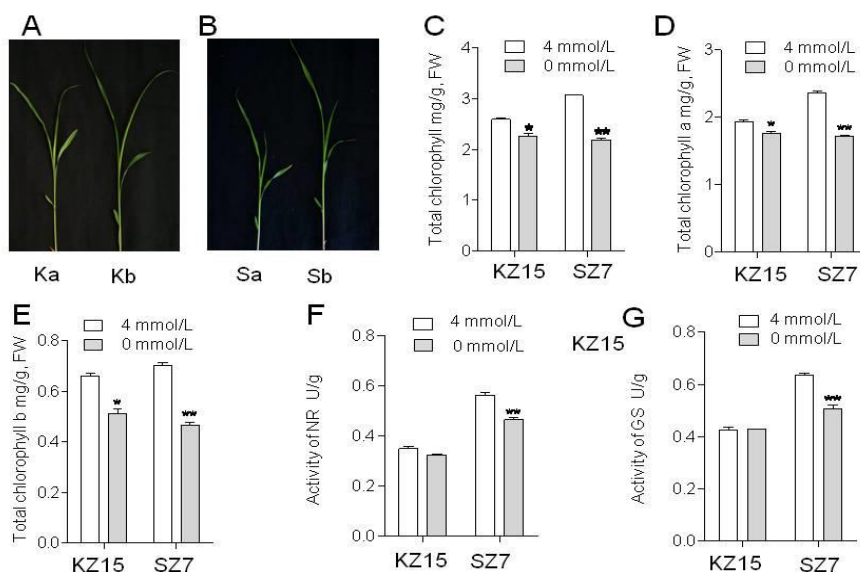
Figure 1. Morphological performance of KZ15 and SZ7 measured under low-N treatment and non-stress concrol nitrogen concentration treatment. (A, B) Phenotype of KZ15 and SZ7 under low-N treatment. (C) Total chlorophyll content. (D) Chlorophyll a content. (E) Chlorophyll b content. (F) Activity of NR. (G) Activity of GS. (*P < 0.05; **P < 0.01). The experiment was performed using three biological replicates, and differences were statistically analyzed using Student's t-test (*P<0.05, **P<0.01). Bars indicate standard error of the mean

Summary of transcriptomic profiling sequencing

Both full-N and low-N treatments were used to analyze the response of sorghum seedlings to nitrogen stress. Twelve libraries were constructed and sequenced. After removing low-quality reads, an average of 24.09 Mb (range of 24.04 to 24.11 Mb) clean reads were obtained in each library, in which more than 86.27% of clean reads per library could be mapped to the reference sorghum genome (*Table 1*). The Q30 values were higher than 90%, which indicated that the sequencing quality was good. The GC content of 12 samples ranged from 52.11% to 55.24%. This result indicated that the sequencing data could be used for further analysis.

Special and common low-N-responsive DEGs between KZ15 and SZ7

To examine the special and common DEGs between KZ15 and SZ7 under low-N treatment, a Venn diagram were constructed. Many common DEGs were identified in KZ15 and SZ7. In total, 793 DEGs were common low-N-responsive genes (*Fig. 2A*),

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among which 277 were upregulated (*Fig. 2B*) and 486 were downregulated (*Fig. 2C*). Under low-N treatment, 404 DEGs were found specifically upregulated in KZ15 and 762 DEGs were specifically upregulated in SZ7. In addition, 642 and 1132 DEGs were specifically downregulated in KZ15 and SZ7, respectively (*Fig. 2A, 2B*).

Table 1. Categorization and abundance of transcripts detected with RNA-seq

Sample	Total Raw Reads (Mb)	Total Clean Reads (Mb)	Clean Reads Q30 (%)	Mapping Ratio to genome	Mapping Ratio to genes	GC (%)
ka_1	24.14	24.11	90.34	94.58%	86.57%	53.21
ka_2	24.14	24.10	90.95	94.64%	86.58%	53.47
ka_3	24.14	24.11	90.88	94.37%	86.27%	52.11
kb_1	24.14	24.07	91.42	94.70%	86.99%	54.23
kb_2	24.14	24.11	91.28	94.83%	87.26%	53.85
kb_3	24.14	24.11	91.52	94.88%	87.35%	53.75
sa_1	24.14	24.11	91.08	94.96%	86.91%	53.11
sa_2	24.14	24.11	90.73	95.20%	87.28%	52.95
sa_3	24.14	24.04	90.71	94.96%	86.91%	55.24
sb_1	24.14	24.07	91	94.83%	86.75%	54.73
sb_2	24.14	24.08	90.79	95.20%	87.14%	55.31
sb_3	24.14	24.08	90.7	94.75%	86.51%	54.53
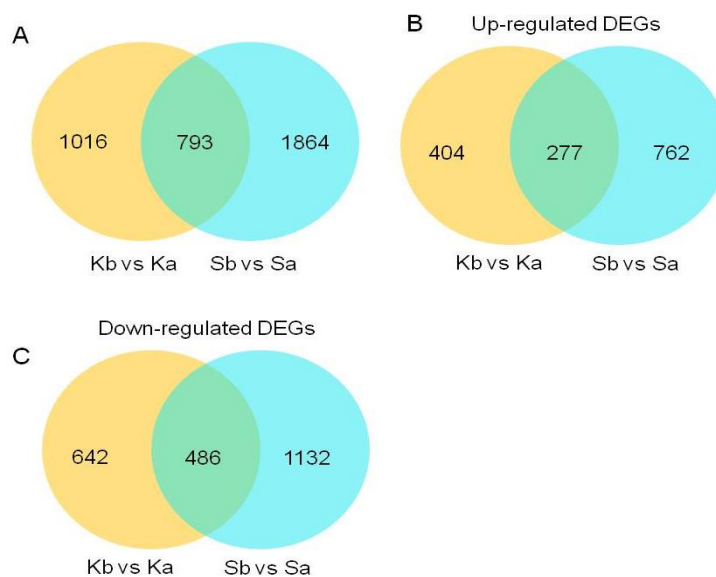
Figure 2. Venn diagrams of differentially expressed genes (DEGs) after nitrogen deficiency., (A).Venn diagram showing the common DEGs between KZ15 and SZ7., (B).Venn diagram showing the special and common up-regulated DEGs between KZ15 and SZ7., (C).Venn diagram showing the special and common down-regulated DEGs between KZ15 and SZ7

GO enrichment of DEGs

The DEGs were categorized into 46 functional groups in KZ15 and SZ7. According to the significance and reliability, GO entries with significant enrichment were screened and simplified. Twenty-six GO entries with the highest enrichment significance were screened out in KZ15 (*Table 2*), and 11 GO items with the highest significance were selected in SZ7 (*Table 3*). In biological process, GO terms related to metabolic process were the most enriched both in tolerant cultivar KZ15 (513) and sensitive cultivar KZ15

(731). DEG transcripts associated with stress responses included drug decomposition process in sensitive genotypes. DEGs associated with the metabolic process of pigmentation and glutathione were enriched in tolerant genotypes.

Table 2. GO analysis of genes specifically expressed in KZ15 after N-starvation

GO term	Ontology	Description	Gene Number	P-value
GO:0009522	C	Photosynthetic system I	10 of 720	0.00022
GO:0005576	C	Extracellular domain	64 of 720	0.00025
GO:0009521	C	Photosynthetic system	11 of 720	0.0439
GO:0016491	F	Oxidoreductase activity	179 of 944	1.97E-11
GO:0003824	F	Catalytic activity	663 of 944	6.69E-06
GO:0048037	F	Cofactor binding	114 of 944	9.42E-06
GO:0046527	F	Glucose transferase activity	38 of 944	2.51E-05
GO:0080043	F	3- Quercetin Glucosyl transferase activity	27 of 944	2.92E-05
GO:0080044	F	7- Quercetin Glucosyltransferase activity	27 of 944	2.92E-05
GO:0046906	F	Tetrapyrrol binding	62 of 944	0.00015
GO:0035251	F	UDP- Glucose transferase activity	31 of 944	0.00049
GO:0016168	F	Chlorophyll binding	8 of 944	0.00787
GO:0020037	F	Heme binding	54 of 944	0.01346
GO:0004364	F	Glutathione transferase activity	17 of 944	0.02068
GO:0042440	P	Pigment Metabolism	18 of 715	6.47E-05
GO:0019748	P	Secondary metabolic process	40 of 715	0.00027
GO:0015994	P	Metabolism of chlorophyll	11 of 715	0.00087
GO:0006778	P	Metabolism of Porphyrin-containing Compounds	14 of 715	0.00251
GO:0051186	P	Cofactor metabolic process	60 of 715	0.00625
GO:0006749	P	Glutathione Metabolism	18 of 715	0.01025
GO:0033013	P	Tetrapyrrol Metabolism	14 of 715	0.01182
GO:0009765	P	Photosynthesis, Light Harvest	9 of 715	0.01922
GO:0018298	P	Protein chromophore linkage	8 of 715	0.02056
GO:0009407	P	Toxin decomposition process	11 of 715	0.02232
GO:0090487	P	Secondary metabolite decomposition process	11 of 715	0.02232
GO:0046148	P	Pigment biosynthesis process	12 of 715	0.02666

C: Cellular component; P: Biological process; F: Molecular function

Table 3. GO analysis of genes specifically expressed in SZ7 after N-starvation

GO term	Ontology	Description	Gene Number	p-value
GO:0005576	C	Extracellular domain	87 of 1111	0.00114
GO:0009522	C	Photosynthetic system I	11 of 1111	0.00263
GO:0009521	C	Photosynthetic system	16 of 1111	0.00485
GO:0016491	F	Oxidoreductase activity	239 of 1407	3.12E-10
GO:0046906	F	Tetrapyrrol binding	90 of 1407	8.89E-07
GO:0048037	F	Cofactor binding	156 of 1407	9.07E-06
GO:0020037	F	Heme binding	83 of 1407	2.19E-05
GO:0003824	F	catalytic activity	951 of 1407	0.00074
GO:0004497	F	Monooxygenase activity	55 of 1407	0.02333
GO:0042737	P	Drug decomposition process	47 of 1019	0.00837
GO:0051187	P	Cofactor catabolism	35 of 1019	0.01595

C: Cellular component; P: Biological process; F: Molecular function

In molecular function ontology, catalytic activity was the term which was enriched and most abundant in both KZ15 (633) and SZ7 (951). The terms oxidoreductase activity, cofactor binding, tetrapyrrole binding and heme binding were found in both KZ15 and SZ7. GO terms belonging to molecular functions such as glutathione transferase activity, chlorophyll binding, glucose transferase activity, 3-quercetin

glucosyl transferase activity, and UDP-glucose transferase activity were enriched in KZ15. With respect to cellular component ontology, 64 genes were enriched in the extracellular region, followed by the photosynthetic pathway, suggesting that genes differentially expressed in the photosynthetic system and extracellular region of KZ15 may play an important role in low nitrogen tolerance. In SZ7, the most significant Go term was extracellular domain (87 genes). The other two GO terms (Photosynthetic system I and photosynthetic system) were related to the photosynthetic system.

Functional enrichment of significant genes

In this study, KEGG pathway enrichment analysis of DEGs of KZ15 and SZ7 under nitrogen starvation were carried out. In KZ15, a total of 1526 genes were found to be enriched in 124 pathways, which were divided into five branches: cell process, genetic information processing, environmental information processing, metabolism, and biological system. The results showed that nitrogen starvation had certain effects on the biosynthesis of secondary metabolites, carbohydrates, amino acids, lipids, terpenoids, ketones, cofactors, vitamins, plant signal transduction, membrane transport, protein folding, sorting, degradation, and DNA transcription. In addition, there were 125 DEGs that were related to environmental adaptation. In this study, the top ten pathways with the highest enrichment level were analyzed (*Table 4*). We found that the difference of nitrogen treatments caused changes of genes related to secondary metabolites biosynthesis, porphyrin metabolism, chlorophyll metabolism, photosynthetic antenna protein pathways, and other pathways, suggesting that these substances may be related to the absorption or metabolism of nitrogen in KZ15.

Table 4. Top 10 KEGG pathway in KZ15 after N-starvation

No.	Pathway ID	Pathway	DEGs genes	Pvalue	Qvalue
1	ko01110	Secondary Metabolites Biosynthesis	283	6.83E-15	8.47E-13
2	ko00860	Porphyrin and chlorophyll metabolism	19	2.70E-07	1.67E-05
3	ko00196	Photosynthetic antenna protein	8	9.05E-06	3.74E-04
4	ko01100	Metabolic pathway	380	1.65E-05	4.31E-04
5	ko00250	Metabolism of alanine, aspartic acid and glutamic acid	16	1.74E-05	4.31E-04
6	ko00220	Arginine biosynthesis	15	2.15E-05	4.44E-04
7	ko00941	flavonoid biosynthesis	22	6.12E-05	1.08E-03
8	ko04712	Plant rhythm	31	0.000188563	2.92E-03
9	ko00350	Tyrosine metabolism	15	0.000289618	3.67E-03
10	ko00908	Zeatin biosynthesis	10	0.000301345	3.67E-03

In SZ7, a total of 1694 genes were found to be enriched in 119 pathways. The results showed that nitrogen treatment had certain effects on the metabolism of carbohydrates, secondary metabolites, amino acids, lipids, terpenoids, ketones, cofactors, microorganisms, plant signal transduction, membrane transport, and DNA transcription and translation. A total of 161 DEGs were related to environmental adaptation. In this study, the top ten pathways with the highest enrichment level were analyzed (*Table 5*). We found that the difference of nitrogen level in SZ7 caused the different expression of genes related to the biosynthesis of secondary metabolites, monoterpenes, carotenoids, content of amino acids, starch, and sucrose, suggesting that these substances might be related to the metabolism of nitrogen uptake of SZ7.

Table 5. *Top 10 KEGG pathway in SZ7 after N-starvation*

No.	Pathway ID	Pathway	DEGs genes	Pvalue	Qvalue
1	ko01110	Secondary Metabolites Biosynthesis	344	5.12E-07	6.46E-05
2	ko00902	Monoterpene biosynthesis	9	7.72E-05	4.86E-03
3	ko00906	carotenoid biosynthesis	18	0.000176	7.40E-03
4	ko00904	Diterpenoid biosynthesis	18	0.000853	2.15E-02
5	ko00350	Tyrosine metabolism	18	0.000853	2.15E-02
6	ko00250	Metabolism of alanine, aspartic acid and glutamic acid	16	0.001541	3.24E-02
7	ko00500	Starch and Sucrose Metabolism	67	0.002004	3.34E-02
8	ko01100	Metabolic pathway	519	0.002124	3.34E-02
9	ko00950	Biosynthesis of Isoquinoline Alkaloids	13	0.003257	4.56E-02
10	ko00909	Biosynthesis of sesquiterpenes and triterpenes	11	0.00804	1.01E-01

Genes involved in nitrogen metabolism and glutathione metabolism

The results of GO enrichment analysis indicated the common low-N responses on nitrogen metabolism and the processes of glutathione metabolism in both KZ15 and SZ7. Nitrogen metabolism was composed of nitrogen transport and nitrate assimilation. In total, 16 genes participated in nitrogen metabolism, including nitrate reductase, ferredoxin-nitrite reductase, ureide permease 2, chloride channel protein and the NRT1/ PTR FAMILY (NRT2.2, NRT2.3, NRT3.1, NRT4.3, NRT4.4, and NRT6.3), which were all upregulated in both KZ15 and SZ7 under N-starvation. Only five genes (IN2-1, NRT4.3, NRT6.3, NRT8.3, and NRT8.5) encoding nitrate transport proteins were downregulated after N-starvation (*Figure 3A*).
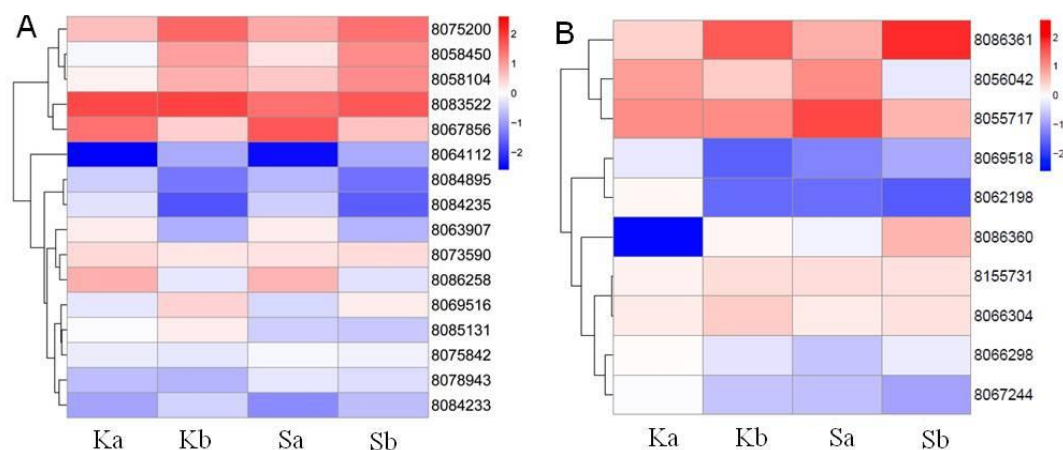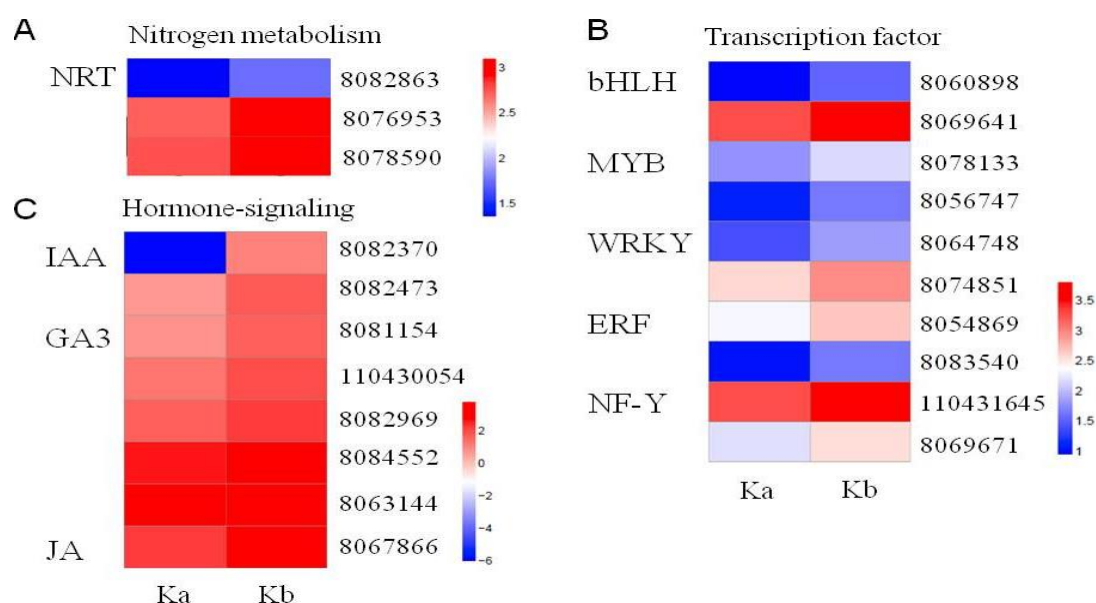

Figure 3. *Heatmap of differentially expressed genes (DEGs) related to Nitrogen metabolism (A) and Glutathione metabolic (B) in KZ15 and SZ7 under N-starvation*

Although similar expression pattern of genes involved in nitrogen metabolism existed in both varieties under N-starvation, the gene expression pattern in cell redox homeostasis was distinct. Among these, eight DEGs mapped to the cell redox homeostasis pathway and glutathione metabolism was found to be upregulated in KZ15 and SZ7 under N-starvation. Only two DEGs had the opposite expression (*Figure 3B*).

DEG transcripts abundant in KZ15 under N-starvation

Genes that are differentially expressed in only one variety should be important for the phenotypic differences between KZ15 and SZ7 under N-starvation. A total of 389 genes that were especially up-regulated in KZ15 were functionally involved in low-N tolerance. The expression of DEGs related to nitrogen metabolism and defense response was identified, which included genes associated with nitrate uptake and assimilation, transcriptional regulation, hormone-signaling, and oxidative stress. The results of RNA-seq for known nitrogen transport and assimilation genes indicated that N-stress may increase the abundance of genes encoding high affinity nitrate transporters in tolerant cultivars KZ15. For example, genes encoding the nitrate transporters NRT2.4, NRT3.1, and NRT4.5 were more abundant in the tolerant genotype KZ15 than SZ7 (*Figure 4A*).

Figure 4. *Heatmap of differentially expressed genes(DEGs) related to low-N tolerance responses in KZ15. (A) Nitrogen metabolism. (B) Transcription factors*

Transcription factors are important in molecular mechanisms regulating growth, developmental processes, and responses to biotic or abiotic stress. In this study, a total of ten transcription factor (TF) encoding genes were especially upregulated in KZ15 under N-starvation. These transcription factors included the WRKY, MYB(myeloblastosis), bHLH(b helix–loop–helix) , ERF (ethylene responsive factor), and NF-Y(nuclear factor-Y) transcription factor families (*Figure 4B*). In addition, several genes associated with hormone-signaling pathways were upregulated in the tolerant genotype KZ15 under N-starvation, such as auxin responsive GH3(Gretchen Hagen 3) gene, auxin-responsive protein IAA30, gibberellin receptor GID1(Gibberellin insensitive dwarf 1), gibberellin 2-oxidase, and jasmonate ZIM (Zinc finger gene) domain-containing protein (*Figure 4C*). DEGs involved in oxidative stress response and flavonoid metabolites were also upregulated in KZ15 under N-stress.

Discussion

Changes of physiology and expressing genes in sorghum under low-N stress

The results of this study showed that the DEGs in chloroplast of SZ7 were significantly more abundant than that of KZ15. When nitrogen was deficient, physiological analysis also showed that the chlorophyll content of SZ7 was significantly lower than that of the normal nitrogen application, while the chlorophyll content of KZ15 did not change significantly under different nitrogen treatments. The activity of NR and GS decreased under low nitrogen, but the decrease of KZ15 was lower than that of SZ7. The decrease of low nitrogen tolerance sorghum was lower than that of low nitrogen sensitivity sorghum. The results of this study are consistent with previous studies on maize (Presterl et al., 2010), wheat (Han et al., 2007; Xiong et al., 2016), and rice (Zeng et al., 2007).

The results of RNA-sequencing revealed a series of DEGs between KZ15 and SZ7. To understand the molecular mechanisms of these two sorghum cultivars with significantly different nitrogen uptake levels, we found that 681 genes and 1039 genes were upregulated in KZ15 and SZ7 without nitrogen application, respectively, and the number of downregulated genes were 1128 and 1618, respectively. As an N^+ starvation sensitive genotype, the results showed that the DEGs of SZ7 were significantly more abundant than those of KZ15 in the process of organic nitrogen biosynthesis and nitrogen metabolism, which is similar to previous studies in maize and Arabidopsis thaliana (Gallais and Hirel, 2004). We also found that both cultivars were involved in photosynthesis and protein differential expression. Similar phenomena have been found in the gene expression profile of Arabidopsis thaliana (Himanen et al., 2004).

Special and common Low-N-responsive DEGs in sorghum under low-N stress

In this study, many DEGs were identified in both sorghum varieties which may be a response to low-N stress. Previous studies have shown that many DEGs associated with high affinity nitrate transporters (NRT2.2, NRT2.3, NRT2.5, and NRT2.6) were identified in low-N tolerant sorghum varieties (Gelli et al., 2014). Consistent with this, we found that the transcript encoding nitrate transporters NRT2.4, NRT3.1, and NRT4.5 were abundant in the tolerant genotype KZ15 compared to SZ7. We also found that the NRT1/ PTR family (NRT2.2, NRT2.3, NRT3.1, NRT4.3, NRT4.4, and NRT6.3) was upregulated in both KZ15 and SZ7 under N-starvation.

It has been reported that SiMYB3 takes part in the growth and development of lateral roots and elongation of main roots mediated by low nutritional stress in millet; it also plays an important regulatory role in the signaling pathways of nitrogen, phosphorus, and potassium (Hu et al., 2015). A total of 235 MYB transcription factors were found in this study. Two MYB transcription factors were especially upregulated in KZ15 under different nitrogen treatments, indicating that MYB transcription factors may play an important role in the process of low-nitrogen stress response in sorghum.

Conclusion

To analyze the molecular mechanism of sorghum response to low nitrogen, the phenotypic and transcript profiling of different resistant varieties were studied. The results showed that the content of chlorophyll and the activity of NR and GS of SZ7

were more sensitive than that of KZ15 under low-N stress. A total of 1809 and 2657 DEGs were identified from KZ15 and SZ7.

We suggest that MYB transcription factors may play an important role in regulating the expression of genes related to nitrogen stress response. The ERF, bHLH, WRKY, and NF-Y families of transcription factors could also be involved in the response to low nitrogen tolerance in sorghum. In the tolerant cultivar KZ15, 389 upregulated genes were found functionally involved in low-N tolerance. These 389 genes are mainly involved in nitrogen metabolism, defense response, oxidative stress response, and flavonoid metabolism. We speculate that three nitrate transporter genes (NRT2.4, NRT3.1, and NRT4.5) and ten transcription factors may play an important role in improving the nitrogen use efficiency of sorghum. Overexpression of these genes may improve the tolerance of sensitive sorghum genotypes to nitrogen starvation. The expression patterns and regulation mechanisms of these genes associated with the tolerance to low nitrogen will be analyzed in future. These genes can also be used as candidate genes for improving sorghum tolerance to low nitrogen by genetic engineering, and lay a foundation for the breeding of new sorghum varieties with low nitrogen tolerance.

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